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Susan M. Cannon  
Printed name of person mailing correspondence

  
Signature of person mailing correspondence

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mark C. Fishman et al.	Art Unit:	1634
Serial No.:	09/759,508	Examiner:	Jehanne E. Souaya
Filed:	January 12, 2001	Customer No.:	21559
Title:	Methods for Diagnosing and Treating Heart Disease		

Commissioner for Patents  
Washington, D.C. 20231

DECLARATION OF XIAOLEI XU, PH.D., UNDER 37 C.F.R. § 1.131

I declare:

1. I am an inventor of the subject matter that is described and claimed in the above-captioned patent application.
2. The enclosed Exhibit is a copy of pages from my laboratory notebook, which show that my co-inventor and I had determined that the pickwick mutation, which is characterized by a weak heartbeat, is in the titin gene. In particular, we found that certain zebrafish sequences that we had identified as being in the pickwick locus were homologous to known titin sequences. These pages are dated prior to the August, 1999 publication date of Satoh et al. (Biochem. Biophys. Res. Com. 262:411-417, 1999).

3. All statements made herein of my own knowledge are true, and all statements made on information and belief are believed to be true, and further, these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: 11/11/02



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pickwick

positional cloning

499

100% marker

use 500 ng / well.

50M

10789

(8 RE / 1000)

→ 1 YAC

connection

500 embryo

→ 3 YAC. by primers

from B'U TR. of connexin  
as superpool.

I

was on <sup>140</sup> use the primer pair to screen the 8 plate pool

Total 24 PCR reaction.

Genomic DNA 1 675 50X. 3 ~~8~~ /  $\lambda$  use 3  $\lambda$ 

$$\begin{array}{r} 12 \\ 8 \\ \hline 20 \end{array}$$

order

cas → enable.

35 cycle

x90

25  $\lambda$ 

Template

4  $\lambda$ (10 ng /  $\lambda$ )

26.

10X A

2.5  $\lambda$ 

22.5

25 mM dNTP

0.1  $\lambda$ 

9

17.8

7

90

1602

→ 6.

primer

0.25  $\lambda$ 

20 mM

22.5

- primer

0.5

22.5

Tag

0.1  $\lambda$ 

9

Hind III

17.8  $\lambda$ 

1602

25  $\lambda$

1. IVF fish do not give eggs out eggs. try

next week. select strong fish!

try with my m88 w/ fish although AB/TL background. at least. get something.

2.

Titin

picknick could be titin. ech1256 show high homology with fish (connection). which makes sense.

1. Z 8363 scan all mutants to identify recombinants.

> 500 embryos. confirm with Z 2003 / about said ID'd.

2. design primers from af 036148. do ~~PCR~~ PCR

together with ech1256 against Y5, Y6. hope to pick

up the right side about Y5T3, Y6T3

3. Compare human / mouse / chick / titin sequence. design primer pairs against the 27 kb cDNA conserved region

① put into RH map. confirm its identity!

② PCR against Y5, Y6.

③ isolate PAC. & get the inton. 3' UTR region

and then design primers for SSCP

~~PCR~~

1. Got embryos for m10624 (their parents are here!)  
m686.9 x TL07 (two pairs)

m10104

m521A (1 # are low)

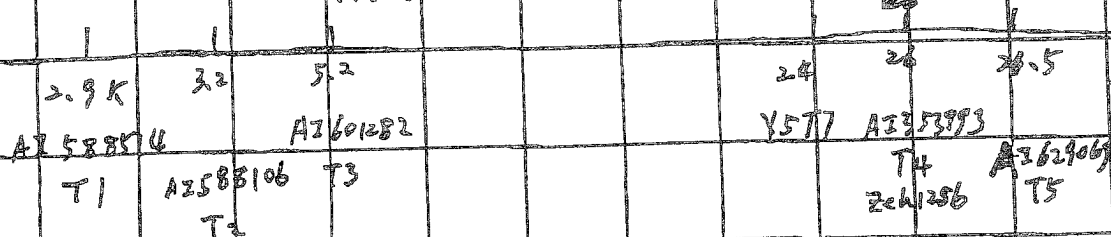
mP18a (TL allele)

Today bleached five out of 6 except m521A  
next Tuesday put them into system.

Tomorrow look at phenotype

More good news!

1. from EST project. 4 were titin zebrafish version!
2. Y5T7 and ~~Y5T7~~ is titin homologue!
3. Best of all. they represent different portions of P-titin



4. according to sequence alignment of Y5T7 the titin gene in chromosome should be

